Claims

1. A method for inducing cardiomyocyte differentiation comprising the steps of:
(i) providing at least one pluripotent stem cell;
(ii) contacting the pluripotent stem cell(s) with:
(a) ~~at least one p38 mitogen-activated protein kinase (p38-MAPK) inhibitor~~ SB 203580 (4-[4-(4-fluorophenyl)-2-(4-methylsulfinylphenyl)-1H-imidazol-5-yl]pyridine);
(b) ~~at least one immunosuppressant~~ cyclosporin-A (CSA); and
(c) ~~at least one Wnt modulator~~ IWP4 (2-(3,4,6,7-tetrahydro-3-(2-methoxyphenyl)-4-oxothieno[3,2-d]pyrimidin-2-ylthio)-N-(6-methylbenzo[d]thiazol-2-yl)acetamide); in the order listed.

2. The method according to claim 1, wherein the pluripotent stem cell(s) comprises isolated pluripotent stem cell(s).

3. The method according to claim 1 or 2, wherein the pluripotent stem cell(s) comprises induced pluripotent stem cell(s) or embryonic stem cell(s).

4. The method according to any one of the preceding claims, wherein the pluripotent stem cell(s) is part of an embryoid body.

5. The method according to claim 4, comprising the steps of:
(i) inducing pluripotent stem cells to form at least one embryoid body;
(ii) contacting the embryoid body with:
(a) ~~at least one p38 mitogen-activated protein kinase (p38-MAPK) inhibitor~~ SB 203580 (4-[4-(4-fluorophenyl)-2-(4-methylsulfinylphenyl)-1H-imidazol-5-yl]pyridine);
(b) ~~at least one immunosuppressant~~ cyclosporin-A (CSA) and

(c) ~~at least one Wnt modulator~~ IWP4 (2-(3,4,6,7-tetrahydro-3-(2-methoxyphenyl)-4-oxothieno[3,2-d]pyrimidin-2-ylthio)-N-(6-methylbenzo[d]thiazol-2-yl)acetamide); in the order listed.

6. A method for inducing cardiomyocyte differentiation, comprising (i) contacting pluripotent stem cell(s) or a embryoid body with a first composition comprising ~~a p38-MAPK inhibitor~~SB 203580 (4-[4-(4-fluorophenyl)-2-(4-methylsulfinylphenyl)-1H-imidazol-5-yl]pyridine), (ii) contacting the pluripotent stem cell(s) or embryoid body with a second composition comprising ~~a p38-MAPK inhibitor~~ SB 203580 (4-[4-(4-fluorophenyl)-2-(4-methylsulfinylphenyl)-1H-imidazol-5-yl]pyridine), and ~~an immunosuppressant~~cyclosporin-A (CSA) and (iii) contacting the pluripotent stem cell(s) or embryoid body with a third composition comprising ~~a Wnt inhibitor~~IWP4 (2-(3,4,6,7-tetrahydro-3-(2-methoxyphenyl)-4-oxothieno[3,2-d]pyrimidin-2-ylthio)-N-(6-methylbenzo[d]thiazol-2-yl)acetamide); in the order listed.

7. A method for inducing cardiomyocyte differentiation, comprising (i) contacting pluripotent stem cell(s) or a embryoid body with a composition B comprising ~~a p38-MAPK inhibitor~~ SB 203580 (4-[4-(4-fluorophenyl)-2-(4-methylsulfinylphenyl)-1H-imidazol-5-yl]pyridine), (ii) contacting the pluripotent stem cell(s) or embryoid body with a composition C comprising a ~~p38-MAPK inhibitor~~ SB 203580 (4-[4-(4-fluorophenyl)-2-(4-methylsulfinylphenyl)-1H-imidazol-5-yl]pyridine), ~~an immunosuppressant~~ cyclosporin-A (CSA) and ~~a Wnt inhibitor~~IWP4 (2-(3,4,6,7-tetrahydro-3-(2-methoxyphenyl)-4-oxothieno[3,2-d]pyrimidin-2-ylthio)-N-(6-methylbenzo[d]thiazol-2-yl)acetamide) and (iii) contacting the pluripotent stem cell(s) or embryoid body with a composition D comprising ~~a p38-MAPK inhibitor~~ SB 203580 (4-[4-(4-fluorophenyl)-2-(4-methylsulfinylphenyl)-1H-imidazol-5-yl]pyridine), ~~an immunosuppressant~~ cyclosporin-A (CSA) and a Wnt inhibitor IWP4 (2-(3,4,6,7-tetrahydro-3-

(2-methoxyphenyl)-4-oxothieno[3,2-d]pyrimidin-2-ylthio)-N-(6-methylbenzo[d]thiazol-2-yl)acetamide); in the order listed.

8. The method according to claim 7, wherein the composition B additionally comprises ascorbic acid, bone morphogenetic protein (BMP), activin A and/or fibroblast growth factor (FBF).

9. The method according to claim 7 or 8, wherein the composition C additionally comprises ascorbic acid, Noggin, a transforming growth factor β (TGF-β) type I receptor activin receptor-like kinase 5 (ALK5) kinase inhibitor and vascular endothelial growth factor (VEGF).

10. The method according to claim 9, wherein the composition C additionally comprises an epidermal growth factor receptor kinase inhibitor.

11. The method according to any one of claims 7 to 10, wherein the composition D additionally comprises ascorbic acid and vascular endothelial growth factor (VEGF).

12. The method according to claim 11, wherein the composition D additionally comprises an epidermal growth factor receptor kinase inhibitor.

~~13. The method according to any one of the preceding claims, wherein the p38-MAPK modulator comprises SB 203580 (4-[4-(4-fluorophenyl)-2-(4-methylsulfinylphenyl)-1H-imidazol-5-yl]pyridine), BIRB 796 (1-[5-tert-butyl-2-(4-methylphenyl)pyrazol-3-yl]-3-[4-(2-morpholin-4-ylethoxy)naphthalen-1-yl]urea), SB 202190 (4-[4-(4-fluorophenyl)-5-pyridin-4-yl-1,3-dihydroimidazol-2-ylidene]cyclohexa-2,5-dien-1-one, VX-702 6-(N-carbamoyl-2,6-difluoroanilino)-2-(2,4-difluorophenyl)pyridine-3-carboxamide), LY2228820 (5-[2-tert-butyl-4-(4-fluorophenyl)-1H-imidazol-5-yl]-3-(2,2-dimethylpropyl)imidazo[4,5-b]pyridin-2-amine; methanesulfonic acid), VX-745 (5-(2,6-dichlorophenyl)-2-(2,4-~~

~~difluorophenylthio)-6H-pyrimido[1,6-b]pyridazin-6-one) or PH-797804 (3-[3-bromo-4-[(2,4-difluorophenyl)methoxy]-6-methyl-2-oxopyridin-1-yl]-N,4-dimethylbenzamide).~~

~~14. The method according to any one of the preceding claims, wherein the immunosuppressant comprises cyclosporin-A (CSA), tacrolimus or sirolimus.~~

~~15. The method according to any one of the preceding claims, wherein the Wnt modulator comprises IWP4 (2-(3,4,6,7-tetrahydro-3-(2-methoxyphenyl)-4-oxothieno[3,2-d]pyrimidin-2-ylthio)-N-(6-methylbenzo[d]thiazol-2-yl)acetamide), IWP3 (2-(3-(4-fluorophenyl)-3,4,6,7-tetrahydro-4-oxothieno[3,2-d]pyrimidin-2-ylthio)-N-(6-methylbenzo[d]thiazol-2-yl)acetamide), IWP2 (N-(6-Methyl-2-benzothiazolyl)-2-[(3,4,6,7-tetrahydro-4-oxo-3-phenylthieno[3,2-d]pyrimidin-2-yl)thio]-acetamide), pyrvinium (2-[(E)-2-(2,5-dimethyl-1-phenylpyrrol-3-yl)ethenyl]-N,N,1-trimethylquinolin-1-ium-6-amine, BIO ((3Z)-6-bromo-3-[3-(hydroxyamino)indol-2-ylidene]-1H-indol-2-one), CHIR99021 (6-[2-[[4-(2,4-dichlorophenyl)-5-(5-methyl-1H-imidazol-2-yl)pyrimidin-2-yl]amino]ethylamino]pyridine-3-carbonitrile) or XAV939 (2-[4-(trifluoromethyl)phenyl]-1,5,7,8-tetrahydrothiopyrano[4,3-d]pyrimidin-4-one).~~

~~16. A method for inducing cardiomyocyte differentiation, comprising contacting pluripotent stem cell(s) or embryoid body with~~

~~(a) the p38-MAPK inhibitor 4-[4-(4-fluorophenyl)-2-(4-methylsulfinylphenyl)-1H-imidazol-5-yl]pyridine (SB 203580);~~

~~(b) the immunosuppressant cyclosporin-A (CSA); and~~

~~(c) the Wnt modulator IWP4 (2-(3,4,6,7-tetrahydro-3-(2-methoxyphenyl)-4-oxothieno[3,2-d]pyrimidin-2-ylthio)-N-(6-methylbenzo[d]thiazol-2-yl)acetamide).~~

17~~3~~. The method according to any one of the preceding claims; comprising additionally contacting the pluripotent stem cell(s) or embryoid body with at least one Reactive Oxygen Species (ROS) activator.

18~~4~~. The method according to claim 17~~3~~; wherein the ROS activator comprises hydrogen peroxide (H_2O_2)